The biological integrators network (RINBIO) in the Mediterranean sea

The use of artificial cages of mussels for the evaluation of chemical contamination

Experimental protocol

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INTRODUCTION

The main French waters management scheme (SDAGE) of the Rhone/Mediterranea/Corsica basin reinforce the political actions along the French Mediterranean coasts. This is based on a better knowledge of naturals ecosystems and the development of coherent information systems. This has been done using a geographical approach where the French coasts have been delimited in 50 different managements areas covering both sea and land.

The biological integrators network (RINBIO) has been initiated by both the Rhone/Mediterranea/Corsica water agency and IFREMER in order to evaluate the levels of chemical contamination in the dilution areas in each of the 50 defined management zones and to complement the existing networks in monitoring both the alteration of clean and the recovery of altered ecosystems.

The direct measurement of contaminants needs sophisticated and costly methods, with some difficulties for their application to the monitoring networks as for their significance. Bio monitoring is based on the ability of mussels to concentrate chemical contaminants in tissues in relation to their biodisponibility. Strategies are of two types : Some are using wild or cultivated mussels (passive monitoring) while some are using transplanted mussels coming from a pristine site.

Since natural mussels are not always available along the French Mediterranean coast, The RINBIO network is using transplanted mussels for monitoring the contamination

STRATEGY AND AIMS OF MONITORING

The aim of the network is to follow the spatial and temporal variations of contamination levels in the impacted areas in each of the 50 managements zones from the French coast. Taking in account both the potential inputs (freshwater quality, industrials and urban inputs, cultivated lands etc…) and the coastal currents , it was possible to locate homogeneously these 50 areas at a regional scale in relation with contaminants inputs.

Different types of contaminants were considered :

- Trace metals : Lead *(Pb), Cadmium (Cd), Copper (Cu), Mercury (Hg), Zinc (Zn), Chromium (Cr), Nickel (Ni), Arsenic (As).*

- organic compounds *: DDT and métabolites (DDD, DDE), Hexachlorocyclohexan (HCH), γHCH (Lindane) et α HCH, Polychlorobiphényls (*congeners CB28, CB31, CB35, CB52, CB101, CB118, CB138, CB153, CB 180), polycyclic *Aromatic hydrocarbons (*Benzo (b) fluoranthene, Benzo (k) fluoranthene, Benzo (a) pyrène, Benzo (ghi) perylene, Indeno (1,2,3-cd) pyrene, Fluoranthene).

Caging method enables to control the age and sexual condition of samples. Using this method at a large scale is inversely related to the physicochemical and trophic variability of stabulation sites.

The amount of accumulated contaminant is strongly related to the biological cycle, including age and sexual mating stage whereas the site conditions (salinity, temperature, trophic level etc..) are affecting not only bio-disponibility and contaminant speciation but also the metabolism an growth of « targeted » mussels.

If the concentrations in tissues are linked to the ambient concentrations of available contaminants, the bio-accumulation factor (ratio between soft tissues concentration and available contaminants in the water) is dependant on the trophic level . Therefore, comparison between concentrations in tissues in areas with different trophic levels may give the wrong results.

The strategy of the RINBIO network , the field methodology and data management has been thought in order to limit these interferences with effects of trophic level on bio-accumulation .

METHOD

Material

Species

The mussel *Mytilus galloprovincialis* is used because of its properties including:

- Physiology and accumulation processes are well known
- A large geographic distribution in the Mediterranean sea and easy to find
- Species is eurytherm and euryhaline
- Transportation is easy

Constraints

Many criteria have to be followed :

- Mussels must have an homogenous genetic pattern . Since wild young Animals are fixed and grown locally , they are of special interest.
- Mussels must be from the same age, ranging from 18 to 24 months (50 mm shell length), when the metabolism is stable,
- Since decontamination is longer than accumulation process, mussels must originate from a pristine site.

Calibration of animals is done at 19 mm shell high . In normal conditions , this lead to a an homogenous population with a 20-25 % natural mortality . Then, mussels are maintained in bags containing 3 kg and immersed for at least 1 week on the originating site for grapping.

Disposition at sea

Criteria for maintaining mussels at sea includes :
- an inexpensive system
- disposition at sea and recovery must be easy,
- the system must be adapted to local hydrodynamism
- the system must take in account the possibility of fouling and high turbidity-

Two different types of systems are used.:

System at sea (figure 1 and photo 1)

It is a subsurface system immersed at –6m from surface in order to limit resistance and avoid any vandalism.

3 kg of mussels sample Ares maintained in a bag (0,5 m X 0.35 m) of 18 mm mesh with2 rigid tubes (40 mm diameter) along the inferior and superior sides of the bag . A 11 liters main buoy in attached at the top of the bag . and the whole system is maintained on the bottom using a 30 kg lest (old chain) , a 8 mm chain (1 meter) and a polypropylene rope (diameter 7 mm) . The recovery is performed using either visual or acoustic detection.

In order to amplify the signal , one special buoy (Nokalon) of 1 liter is added for :
- locate more precisely the system when using the sonar from the ship
- obtain a specific signal when using the vertical sounder
- catch the system using ropes from the boat without any divers

At each site , duplicates or triplicates are disposed to optimise the number of recovered bags.

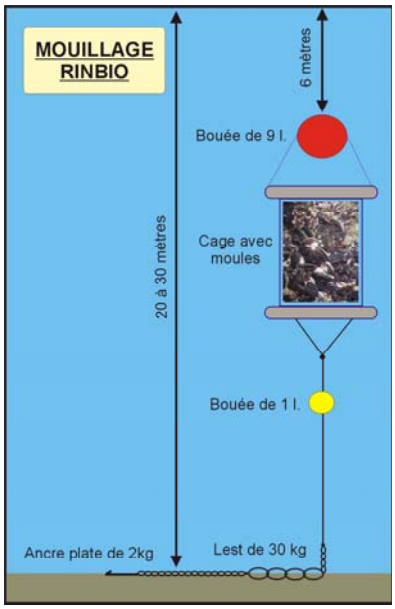


Figure 1 : model of the RINBIO system

Photo 1 : RINBIO bag with its

Disposition of bags in lagoons.

In lagoons , the 3 kg bag is maintained at mid depth , lying (attached) on a table or on a permanent oyster cultivation system

Field exposition

Date and time of immersion

Immersion must be performed during sexual inactivity from April to August. During inactivity period , reserves are high and physiology is constant . In addition contaminant could be lost by mating and spawning ,interfering with accumulation of contaminants…Usually , mussels are immersed for 3 months , from the first half of April to the first half of July.

Nautical support

The support also must be able to work in shallow waters (at the meter scale) , must have a positioning (GPS) system , a sounder and must enable work in windy conditions along the coasts.

Positioning

Mussels are positioned in areas were pollutants are concentrated, taking in accounts the inputs, the effluents and the local currents . The depths must be in the same range for all stations , usually ranging from 10 to 30 m depending on the slopes .

In lagoons, mussels are maintained close to the bottom avoiding sediment and mud

Recovery

At sea, recovery of mussels is performed with divers or **« grapinage** » (photos 2 et 3). The use of a GPS , a sonar and a vertical sounder enables to locate the buoys without any problem. The 2 or 3 lenticular signals at known depths enables to discriminate the mussels amongst the schools, floating debris or thermoclins that are common in the coastal areas. Normally , the whole system is recovered including lests and ropes.

After 15 minutes and the use of divers , if not recovered , the system is considered as lost.

Photos 2 and 3 : recovery of mussels bags with divers

For lagoons, recovery of mussels is performed manually using a small boat

Conditioning of samples

The area used for dissection and conditioning of samples must be clean and must have a fridge and a freezer. Distilled water must be available .

Two strategies can be followed :

1) samples are transported to the laboratory every day

2) samples are conditioned in a following a laboratory-truck every evening.

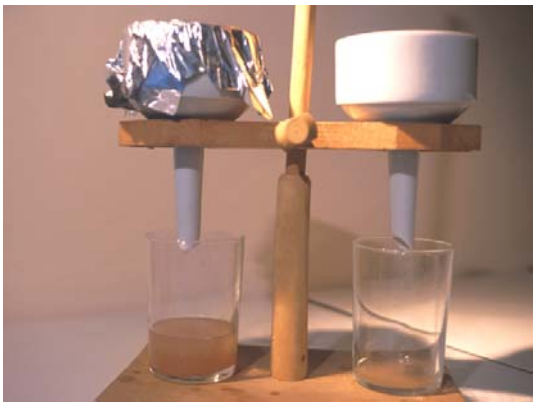
Data concerning biometry are recorded during recovery operations. Mortality is evaluated on board and include mortality occurred during maintenance (20 %). And is a good indicator of life conditions . Shells high enable the evaluation of size homogeneity (15 are randomly measured precisely at the 0.1 mm scale.)

Preparation of beakers.

Elimination of shells must be done carefully with a sterile scalpel avoiding the soft tissues. Byssus must be discarded . water is eliminated putting flesh on a buchner filter during 30 minutes (photographs 4 and 5) . This phase is the most delicate since the animals are exposed to ambient air or diverse projections and may be artificially contaminated. The ambient air must therefore be as clean as possible ideally under a **hotte a flux laminaire** with an aluminium paper on the buchner.

Polyethylene gloves must be used and must be changed for every bag. When a bag has been completed , buchner and scalpels are rinsed with distilled water before re-use.

For each sample , when water has been discarded, a beaker (known weight at 0.1 g) is filled with remaining flesh and covered with aluminium paper. Before closing it with a plastic cap. Beakers are referenced (date , bag , site, nb of mussels etc…) and then freezed



Photos 4 and 5 : preparation of samples

Analysis protocol

Shells and flesh dry weights

Dry weight of the shells and flesh are measured in order to calculate a condition index. In addition when the trophic conditions are different , the results must be corrected before any comparison within sites the ratio mean dry weight of flesh /mean dry weight of shell weight (condition index) takes in account the variability related to the trophic conditions of the various sites.

After recovery , shells from individuals used for chemical analysis are dried at 60°C and weighted . The weight is divided with the number of individuals.
The flesh from beakers is lyophilised and also weighted in the laboratory . As for shells, the weight is divided with the number of individuals to obtain the mean dry weight from each site .

Cd, Pb, Cu, Zn et Hg determination

Traces metals are analysed in mussels using the common methods of absorption or emission spectroscopic methods after specific preparation of samples. An acid treatment of tissues for mineralisation is common to all trace metals in order to destroy the organic matter and to oxidise the metals. Then after cooling, part of the sample is used for cadmium determination , lead, copper and zinc. Cadmium and lead are determined using AAS with a graphic **furnace.** Other metals are analysed either using AAS with thermic atomisation or atomic emission spectroscopy using plasma torch.
The remaining extract is used for mercury determination. Mercury is oxidised in mineral mercury and then measured using atomic fluorescence(Annex 4).

Nickel, chromium and arsenic determination.

As for metals , nickel and chromium are measured , after mineralisation , through GF-AAS or ICP.
Arsenic , as for mercury, is determined with atomic fluorescence.

Organochloride determination.

Chromtography is used for PCB's(polychlorobyphenyls) and PAH (polyaromatic hydrocarbons)
Organochlorides are extracted from mussel flesh using an organic solvent . Then the organic phase is divided in two parts , one for pcbs determination and the other for HAP. PCBs are determined through GC/electron capture when PAH are determined through HPLC/uv detection.

Data processing and interpretation

Using the condition index to correct the contamination in different trophic condition has been already described. This ratio , dry flesh weight/ dry shell weight is a good indicator of the physiologic state (energetic reserves, tissues growth, sexual state…) of certain shellfish. However, this method does not consider the bioaccumulation processes that could be modelised

In the context of the RINBIO network , some correlations some were found between contaminants levels and the physiological parameters of the mussels.
The indicators of the soft tissues growth are significantly correlated to the tissular concentration of most of the contaminants. The condition index (FDW/SDW ; Flesh dry weight /shell dry weight) is the most correlated index amongst all indicators.
Depending in the nature of the contaminant, two different models may explain the relation between contaminant levels and the condition index.

For the first model, tissues concentration is inversely correlated to the condition index. Contaminants concerned with this relation are **Zinc**, **Mercury** , l**Cadmium**, A**rsenic**, **Copper, Nickel** and lead .
For the second model tissue concentration is proportional to the condition index. Contaminants concerned with this relation are **Chromium,** organochlorides (**PCB, DDT, DDD** et **DDE**) and **fluoranthène**.

Depending on contaminants, some sites in supposed contaminated areas don' t follow the linear relation but are significantly above the expected values such as in the example of cadmium , in the following figure 1.

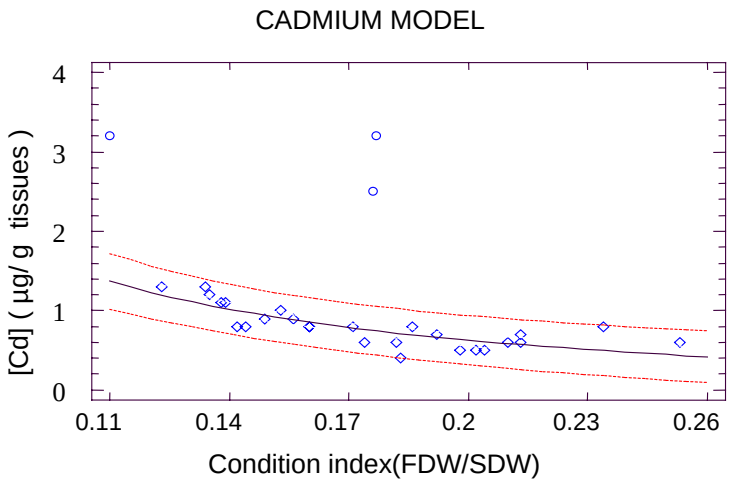


Figure 1 : linear regression model for cadmium. In red is the confidence interval (P< 0.05) . All three stations outside the confience interval are related to the three sites from the Bages lagoon complex(costal lagoon).

For each contaminant, discarding these values from the data bank enables the adjustment of the model which is supposed to indicate the «condition index effect» and enables the characterisation of an average level (background) of the contamination at the RINBIO network scale.

Due to the above described models , measured concentrations may be adjusted to a standard individual which is the mean condition index . Concentrations may therefore be compared at the network scale.

ADVANTAGES AND PROBLEMS OF THE METHODS

Many countries are actually monitoring the contaminant levels using wild mussels (mussel watch) as sentinel species.

Depending on their aims , these programs are limited because of methological constraints affecting the sampling strategy :

- Species are some times absent from the monitored sites

-External factors such as size, season , organ partition of contaminant are a affecting contaminant levels

- Genetic factors may affect the bioaccumulation process.

In a natural population, selection of animals from the same sizes does not imply a same age and heterogeneity linearly affect the size diversity. This can be avoided using transplanted animals originating from the same site.

Artificial caging has been used since 1970's with the following advantages :
- Exposure time can be controlled
- Sites can be chosen independently from the natural occurrence of wild animals
- Samples are more homogenous in terms of originating population , site, size, age ,depth…
- Experiments can be performed with a selected species

The following problems are the most important :

- Logistic must be optimised since animals must be stipulated at sea for a long time
- Climatic conditions such as human factors may affect the maintenance of artificial cages.

Depending on local meteorology, hydrodynamism and depth, technical solutions are existing enabling experiments to be held in good conditions

Even with homogeneous parameters of the transplanted animals, results are linked to the bioaccumulatyon kinetics . Comparisons are more difficult to perform but the use of trophic effects measuring models limits interferences and facilitates interpretations.

FIELD APPLICATION AND OUTLOOKS

Experiments performed in the context of the RINBIO network have demonstrated the interest of using artificial caging of mussels for monitoring the contamination of the Mediterranean sea at local , regional , national and various temporal scales. After a pilot study in 1996, the network is permanently operational . Three experiments have been performed in 1998, 2000 and 2003 with respectively 40, 97 and 106 artificial cages all along the French Mediterranean coast , including coastal lagoons.

Recovery was between 85 and 99% and demonstrate the viability of the method. Moreover , the method with the data process enabled the discovery of new contaminated sites.

Concerning the adjustment model, some research will be necessary to improve their precision, and therefore the evaluation of inter experiments trends. The models will have to be also reconsidered in the context of monitoring of inputs to the shallow waters areas.

Some other experiments have been conducted with success :
- monitoring of effluents from industry dredging activity and water treatment plant.
- EBS (environmental baseline studies) and detection of local contamination.

REFERENCES

Andral B., Stanisiere J. Y.,Sauzade D., Damier E., Thebault H., Galgani F., Boissery P.2004. Monitoring chemical contamination levels in the Mediterranean based on the use of mussel caging. Marine Pollution Bulletin. 49 . 704 – 712.

Andral B., Stanisiere J. Y., Thebault H., Boissery P. 2001. Surveillance des niveaux de contamination chimique et radiologique en Méditerranée basée sur l'utilisation de stations artificielles de moules. Rapport du 36ème congrès de la CIESM, Monaco septembre 2002, volume 36 (1), 107.

Andral B ; 2001. Control de los niveles de contaminacion quimica con bioacumuladores : programa RINBIO. Séminaire de restitution final du Programme INTERREG II C « Vigilancia y control de la contamination marina litoral en el Mediterraneo ». Barcelone 28 – 30 novembre 2001 ;

Andral B., Stanisière J. Y. 2001. Réseau Intégrateurs Biologiques : évaluation de la qualité des eaux basée sur l'utilisation de stations artificielles de moules en Méditerranée : résultats de la campagne 2000. Rapport à l'Agence de l'Eau Rhône Méditerranée Corse ; convention n° 010706.

Andral B., Stanisière J. Y. 2000. Réseau Intégrateurs Biologiques : évaluation de la qualité des eaux basée sur l'utilisation de stations artificielles de moules en Méditerranée : résultats de la campagne 1998. Rapport à l'Agence de l'Eau Rhône Méditerranée Corse ; convention n° 991452 et 992461.

Bergman A .,1993. Concentration of PAH, PCBs and heavy metals in the blue mussel *Mytilus edulis*. 1993 Project Polwad/ Beon Effekt Ministry of Transport, Public Work and Water Management of Netherlands.

Claisse D., Joanny M., Quintin J Y., 1992. Le réseau national d'observation de la qualité du milieu marin (RNO) . Analusis . 6 : 19 - 23.

Comité de Bassin. Schéma Directeur d'Aménagement et de Gestion des Eaux du Bassin Rhône-Méditerranée-Corse. Agence de l'Eau RMC Ed., Lyon (1995).

Denis J., Miossec L., Hénocque Y., Console J.J et Angeli J.P.,1994. Qualification du littoral Méditerranée et cartographie en zones homogènes. Rapport d'étude pour l'Agence de l'Eau RMC, IFREMER Toulon.

Fischer H ., 1984. Cadmium body burden/Shell weight of mussel : a preciseindexfor environmental monitoring. Comm Meet in Count Explor See CM - ICES/E 41 : 1 - 19.

Joanny M., Console J. J., Claisse D., Hénocque Y., 1994. Proposition d'un cahier des charges pour une étude de la qualité du milieu littoral basée sur des intégrateurs biologiques. Proposition finale. Rapport d'étude pour l'Agence de l'Eau RMC, IFREMER Toulon.

Joanny M., Belin, C., Claisse D., Miossec L., Berthomé J. P., Grouhel A., Raffin B., 1993 Qualité du milieu marin littoral - Editions Ifremer.

Lobel P.B and Wright., D.A 1982. Relationship between body zinc concentration and allometric growth measurement in the mussel Mytilus edulis. Mar. Biol. 66 145-150.

O'Connors T. P., 1992. Mussel Watch: recent trends in coastal environmental quality. U. S. Department of Commerce. National Oceanic and Atmospheric Administration.

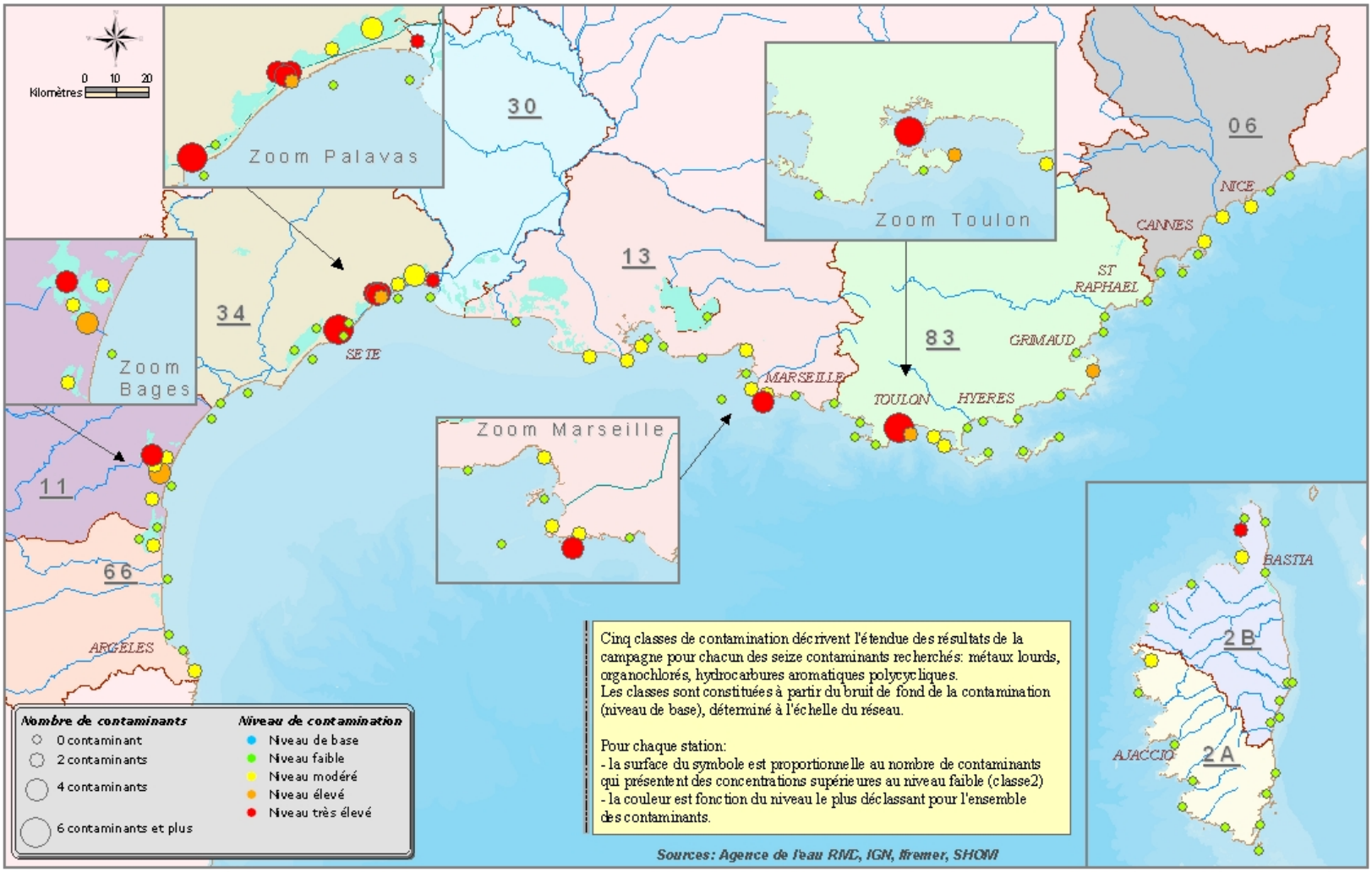
Phillips D. J. H., 1976. The common mussel Mytilus edulis as an indicator of pollution by zinc, cadmium, lead and copper. Effect of environmental variables on uptake of metals. Mar. Biol. 38 : 59 - 69.

Soto M., Kortabitarte M., Marigomez I., 1995. Bioavailable heavy metals in estuarine waters as assessed by metal/shell weight indices in sentinel mussels Mytilus galloprovincialis. Mar. Ecol. Pro. Ser. 125 : 127 - 136.

Thébault H.,Charmasson S., Calmet D., Arnaud M et Henocque Y., 1997. The coastal Mediterranean monitoring network project : radionucleide survey . Radiation Protection Donsimetry, in press.

Wang W.X., Fisher N.S., Luoma, S. N., 1996. Kinetic determination of trace element bioaccumulation in the Mytilus edulis.Mar. Ecol. Prog. 140 : 91 - 113.

http://www.ifremer.fr/envlit/region/reg10paca/rlm.htm#RINBIO



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agence de l'eau rhône méditerranée & corse